Diffusive Resistance, Titratable Acidity, and CO_2 Fixation in Two Tropical Epiphytic Ferns

S. C. WONG and C. S. HEW*

Crassulacean acid metabolism (CAM) is known to occur in many succulent plant species (Ranson & Thomas 1960; Ting et al., 1972; Wolf, 1960). To date, more than 184 plant species have been reported to exhibit CAM features, but none of them are ferns (Szarek, pers. comm.). Recently we reported nocturnal assimilation of CO_2 by *Drymoglossum piloselloides* (L.) Presl, an epiphytic fern (Hew & Wong, 1974). This paper presents further evidence to support our previous findings that certain epiphytic ferns do exhibit characteristics of CAM plants.

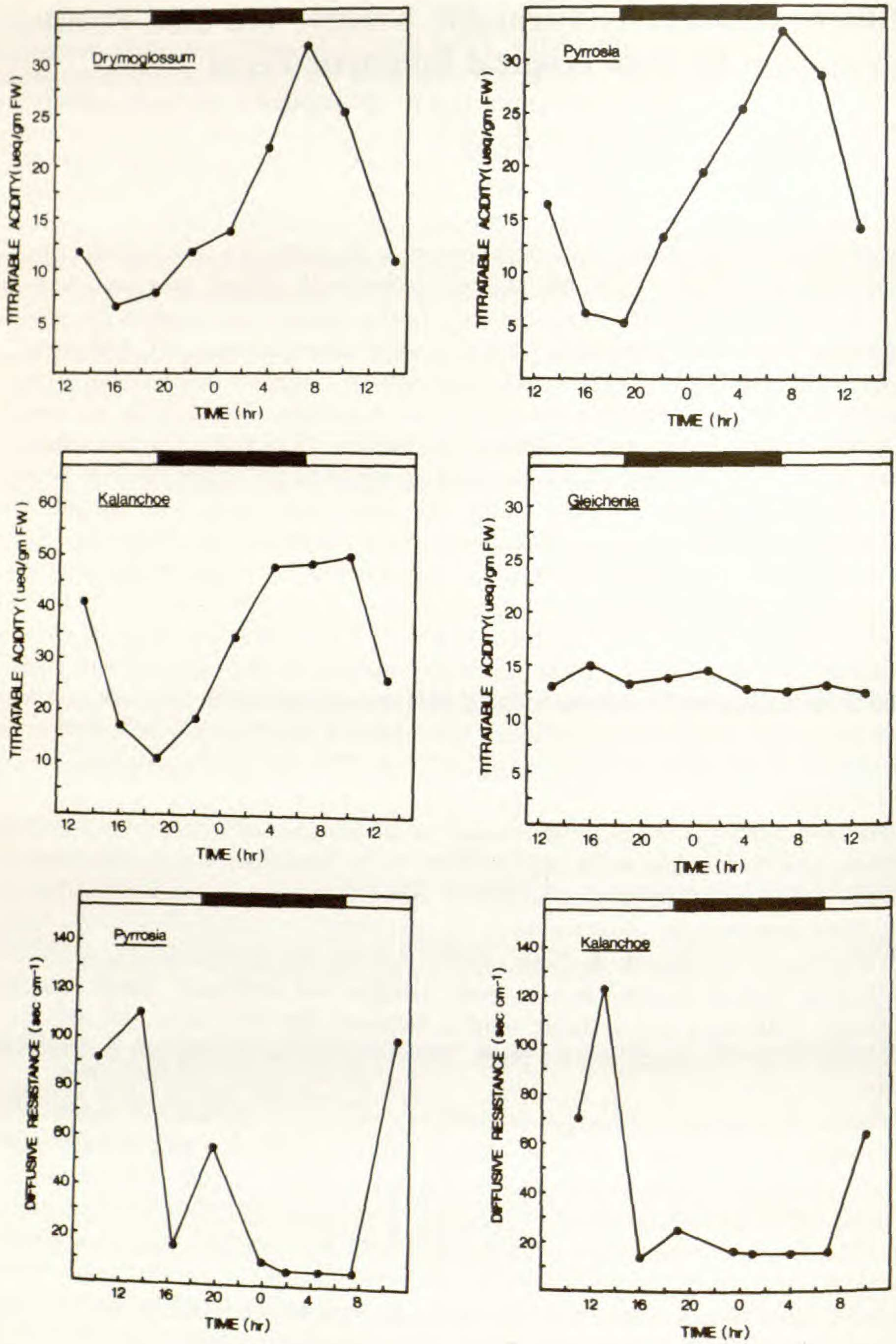
Two epiphytic ferns, *Pyrrosia longifolia* (Burm.) Morton and *Drymoglossum piloselloides,* were chosen for the present investigation. These ferns are found frequently on the lower part of *Acacia* tree trunks. *Gleichenia linearis* (Burm.) Clarke, a terrestial sun fern which has been shown to be a C_3 plant (Hew & Wong, 1974), also was included in the study. All three ferns grow wild around the Nanyang University campus. For comparison purposes, the flowering plant *Kalanchoë pinnata,* a known CAM plant, was also used as experimental material.

For $^{14}CO_2$ fixation studies and determination of titratable acidity, detached leaves or fronds were used; the method for $^{14}CO_2$ fixation has been described previously (Wong & Hew, 1973). Titratable acidity of plant tissues was determined as described by Szarek and Ting (1974), except the leaf or frond extract was titrated to a $pH_7\uparrow$ end point. Diffusive resistance of intact fronds or leaves were measured at three hour intervals using an Li-60 Diffusive Resistance Meter (Li-Cor Limited).

Titratable Acidity.—The diurnal changes in titratable acidity of *Drymoglossum, Pyrrosia,* and *Kalanchoë* were similar (*Figs. 1-3*). Titratable acidity decreased in the light, and at night the acidity increased. The magnitude of dark acidification in these three species was comparable to that previously reported (Bruinsma, 1958; McWilliams, 1970; Szarek & Ting, 1974). Among the three species, titratable acidity was highest in *Kalanchoë*, both in light and darkness. There was no significant difference in titratable acidity between the two ferns. In contrast, *Gleichenia* (*Fig. 4*), which is a C_3 plant, shows no diurnal fluctuation in titratable acidity.

Diffusive Resistance.—Changes in diffusive resistance patterns of intact *Pyrrosia* fronds (*Fig. 5*) and *Kalanchoë* leaves (*Fig. 6*) in the day and at night were similar, with high diffusive resistance in the day and low at night. The values for minimum diffusive resistance (5-15 $sec \cdot cm^{-1}$) and maximum diffusive resistance (100-120 $sec \cdot cm^{-1}$) also were in agreement with that of other succulent plants (Szarek & Ting, 1974; Ting et al., 1972). From the changes in patterns, one could conclude that *Pyrrosia* and *Drymoglossum* stomata were closed in the day and open at night (Nishida, 1963; Ting et al., 1972). A point worth noting is that with the onset of darkness, an increase in diffusive resistance in both *Pyrrosia* and

*Department of Biology, Nanyang University, Republic of Singapore.



FIGS. 1-4. Diurnal changes in titratable acidity of *Drymoglossum, Pyrrosia, Kalanchoë,* and *Gleichenia*. FIGS. 5-6. Diurnal changes in diffusive resistance of *Pyrrosia* and *Kalanchoë*.

Kalanchoë was observed (*Figs. 5-6*). This increase in resistance probably could account for the rapid decrease in CO_2 uptake by thick-leaved orchids when light was turned off suddenly during the course of CO_2 gas exchange determination (Hew, 1976; Wong, 1973).

$^{14}CO_2$ Fixation.—To ascertain the nature of dark acidification in ferns, *Pyrrosia* and *Drymoglossum* fronds were harvested at 3 p.m. and were allowed to fix $^{14}CO_2$ in darkness for various lengths of time. Malate was the only product labelled with radioactive carbon in a short term fixation experiment. The increase in titratable acidity in *Pyrrosia* and *Drymoglossum*, therefore, was due to a massive accumulation of malate, as has been observed in other CAM plants (Bradbeer et al., 1958; Cockburn & McAulay, 1975; McWilliams, 1970; Ranson & Thomas, 1960; Sutton & Osmond, 1972; Ting et al., 1972).

Pyrrosia and *Drymoglossum* are two of the very common tropical epiphytic ferns of exposed or moderately exposed places in Singapore. These ferns are, in fact, closely related (Holttum, 1954). The fronds are fleshy and contain special layers of water storage cells. Also, the lower surface of the frond of both species is covered with stellate hairs, which prevents excessive water loss (Holttum, 1954). As in other plants (Hew, 1976; McWilliams, 1970; Neales et al., 1968; Neales & Hew, 1975; Ting et al., 1972), structural adaptations in ferns are accompanied by physiological changes. From the diurnal changes in diffusive resistance, titratable acidity, and CO_2 fixation, we conclude that *Pyrrosia* and *Drymoglossum* are CAM plants.

LITERATURE CITED

BRADBEER, J. W., S. L. RANSON, and MARY STILLER. 1958. Malate synthesis in crassulacean leaves: I. The distribution of C^{14} in malate of leaves exposed to $C^{14}O_2$ in the dark. Plant Physiol. **33:** 66-70.

BRUINSMA, J. 1958. Studies on the crassulacean acid metabolism. Acta Bot. Neerl. **7:** 531-588.

COCKBURN, W. and A. McAULAY. 1975. The pathway of carbon dioxide fixation in crassulacean plants. Plant Physiol. **55:** 87-89.

HEW, C. S. 1976. Patterns of CO_2 fixation in tropical orchid species. *In* Proc. 8th World Orchid Conference (In press).

———, and Y. S. WONG. 1974. Photosynthesis and respiration of ferns in relation to their habitats. Amer. Fern J. **64:** 40-48.

HOLTTUM, R. E. 1954. Flora of Malaya, Vol. II. Ferns of Malaya. Gov't. Printing Office, Singapore.

McWILLIAMS, E. L. 1970. Comparative rates of dark CO_2 uptake and acidification in the Bromeliaceae, Orchidaceae, and Euphorbiaceae. Bot. Gaz. **131:** 285-290.

NEALES. T. F. and C. S. HEW. 1975. Two types of carbon fixation in tropical orchids. Planta **123:** 303-306.

———, A. A. PATTERSON, and V. J HARTNEY. 1968. Physiological adaptation to drought in the carbon assimilation and water loss of xerophytes. Nature **219:** 469-472.

NISHIDA, K. 1963. Studies on stomatal movement of crassulacean plants in relation to the acid metabolism. Physiol. Plant **16:** 281-298.

RANSON, S. L. and M. THOMAS. 1960. Crassulacean acid metabolism. Ann. Rev. Plant Physiol. **11:** 81-110.

SZAREK, S. R. and I. P. TING. 1974. Seasonal patterns of acid metabolism and gas exchange in Opuntia basilaris. Plant Physiol. **54:** 76-81.

SUTTON, B. G. and C. B. OSMOND. 1972. Dark fixation of CO_2 by crassulacean plants. Evidence for a single carboxylation step. Plant Physiol. **50:** 360-365.

TING, I. P., H. B. JOHNSON, snd S. R. SZAREK. 1972. Net CO_2 fixation in crassulacean acid metabolism plants. *In* C. C. BLACK (ed.). Net carbon dioxide assimilation in higher plants. Proc. Symp. So. Sect. Amer. Soc. Plant Physiol. and Cotton, Inc., Raleigh.

WOLF, J. 1960. Der diurnal Säurerhythmus. Handb. Pflanzenphysiol. **12**(2): 809-889.

WONG, S. C. 1973. A study of photosynthesis and photorespiration in some thin-leaved orchid species. M.S. thesis, Nanyang University, Singapore.

———, and C. S. HEW. 1973. Photosynthesis and photorespiration in some thin-leaved orchid species. J. Singapore Nat. Acad. Sci. **3:** 150-157.